L13 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1991:427250 CAPLUS
DOCUMENT NUMBER: 115:27250
TITLE: Improved permeabilization procedure for flow cytometric detection of internal antigens. Analysis of interleukin-2 production
AUTHOR(S): Labalette-Houache, M.; **Torpier, G.**; Capron, A.; Dessaint, J. P.
CORPORATE SOURCE: Fac. Med., Inst. Pasteur, Lille, 59019, Fr.
SOURCE: Journal of Immunological Methods (**1991**), **138**(2), **143**-53
CODEN: JIMMBG; ISSN: 0022-1759
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cell membrane permeabilizing treatment is described which involves the use of lysolecithin at low concn. in acidic acetate buffer and paraformaldehyde fixation. It preserved well-sepd. scatter cytograms of small and large lymphocytes. The accuracy of the immunochem. detection of internal antigens by flow cytofluorog. was demonstrated by the linear relationship between the percentage of fluorescent cells detected and the proportion of intracellular antigen-contg. cells in mixts. with antigen-neg. cell lines. Cell cycle anal. by dual nuclear staining with propidium iodide and FITC-conjugated Ki-67 antibody recognizing in vitro stimulated human T lymphocytes verified that the proliferating lymphocytes retained their increased light scatter properties after permeabilization. Enumeration of interleukin-2-(IL-2) producing cells by their cytoplasmic immunofluorescence showed that enlarged lymphocytes were the main IL-2-producing cells. This improved permeabilization procedure, by gating small and enlarged lymphocytes sep., makes it possible to det. by two color fluorescence the immunophenotype of activated T cells committed to interleukin prodn.

L17 ANSWER 31 OF 33 SCISEARCH COPYRIGHT 2003 ISI (R)
ACCESSION NUMBER: 92:570611 SCISEARCH
THE GENUINE ARTICLE: JP567
TITLE: THE DETECTION OF INTRACYTOPLASMIC INTERLEUKIN-1-ALPHA, INTERLEUKIN-1-BETA AND TUMOR-NECROSIS-FACTOR-ALPHA EXPRESSION IN HUMAN MONOCYTES USING 2 COLOR IMMUNOFLUORESCENCE FLOW-CYTOMETRY
AUTHOR: DECAESTECKER M P (Reprint); TELFER B A; HUTCHINSON I V; BALLARDIE F W
CORPORATE SOURCE: MANCHESTER ROYAL INFIRM, DEPT MED, RECORDS OFF M3, OXFORD RD, MANCHESTER M13 9WL, LANCS, ENGLAND (Reprint); MANCHESTER ROYAL INFIRM, DEPT IMMUNOL, MANCHESTER M13 9WL, LANCS, ENGLAND; UNIV MANCHESTER, MANCHESTER M13 9WL, LANCS, ENGLAND
COUNTRY OF AUTHOR: ENGLAND
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (**18 SEP 1992**) Vol. 154, No. 1, pp. 11-20.
ISSN: 0022-1759.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 21

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two colour flow cytometry was used to analyse in situ cytokine expression by human monocytes, Whole blood was cultured in siliconised glass bottles, with or without E. coli lipopolysaccharide (LPS), for various times, and the mononuclear cells (MNCs) then exposed to a variety of permeabilisation procedures prior to flow cytometric analysis. Paraformaldehyde (PF)/saponin fixation preserved cellular morphology, and caused a reproducible degree of permeabilisation (estimated by propidium iodide inclusion: mean 94%, range 86-99% (n = 33)). After fixation with 4% PF and permeabilisation with 1% saponin at 0-degrees-C in PBS containing 20% human serum, MNCs were incubated with phycoerythrin(PE)-conjugated mouse anti-CD14 (monocyte phenotype) and polyclonal rabbit anti-human interleukin-1alpha (IL-1alpha), IL-1beta, tumour necrosis factor alpha (TNF-alpha), or control rabbit IgG. Binding of rabbit antibodies was detected using goat anti-rabbit IgG fluorescein isothiocyanate (FITC). FITC fluorescence was increased in CD14 PE positive cells with the three anti-cytokine antibodies following LPS stimulation, compared with controls. There was a reproducible dose related response in monocyte IL-1beta and TNF-alpha expression following LPS stimulation, with early peaks in TNF-alpha (2 h), compared with IL-1beta (4 h), and IL-1alpha (12 h). Specificity of this cytokine detection system was confirmed by inhibition studies using the corresponding recombinant human cytokines, by an absence of staining in CD14 negative or unpermeabilised MNCs, and by the characteristic cytoplasmic localisation of the different cytokines visualised with UV immunochemistry, Hence, the methods described here provide a reproducible, semiquantitative and specific assay for the detection of cell associated monokines. The technique may be applicable to the analysis of a variety of different cytokines in other phenotypically defined cell populations.

L17 ANSWER 25 OF 33 SCISEARCH COPYRIGHT 2003 ISI (R)
ACCESSION NUMBER: 94:384922 SCISEARCH
THE GENUINE ARTICLE: NQ803
TITLE: FLOW CYTOMETRIC DETECTION OF ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODIES
AUTHOR: YANG Y H; HUTCHINSON P; LITTLEJOHN G O; BOYCE N (Reprint)
CORPORATE SOURCE: MONASH MED CTR, DEPT CLIN IMMUNOL, 246 CLAYTON RD, CLAYTON, VIC 3168, AUSTRALIA (Reprint); MONASH MED CTR, DEPT CLIN IMMUNOL, CLAYTON, VIC 3168, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (**03 JUN 1994**)
Vol. 172, No. 1, pp. 77-84.
ISSN: 0022-1759.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 27

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An intracytoplasmic immunofluorescence staining technique which allows the detection and quantification of anti-neutrophil cytoplasmic autoantibodies (ANCA) by flow cytometry is described. A polymorph neutrophil population from human peripheral blood was used in this study as indicator cells. These were fixed and permeabilized by paraformaldehyde, Tween 20 and saponin, to allow ANCA in the patients sera to reach their intracellular antigen targets. The numbers of indicator cells remained unaltered by the permeabilization protocol and no cell aggregation or loss of intracellular antigenicity was observed. An excellent agreement (91% (207/228)) between ANCA detection by immunofluorescence microscopy (IF) and flow cytometry was noted. Compared with IF assay, the flow cytometric method has a sensitivity of 93% (42/45) and a specificity of 90% (165/183). Although not able to discriminate between P-ANCA or C-ANCA, this flow cytometric method has the advantage of providing an objective, reproducible and quantitative measure of ANCA, which makes it an ideal technique for screening of patients sera for ANCA reactivities.